This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

1. REGISTRATION NO. 84-R-0007 **CUSTOMER NO.** 1086

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

COLORADO SERUM COMPANY
P. O. BOX 16428
DENVER, CO 80216
(303) 295-7527

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS*(sites)*

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use APHIS FORM 7023A)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs	-	-	-	-	-
5. Cats	-	-	-	-	-
6. Guinea Pigs	2	371	328	1482	2181
7. Hamsters	107	404	0	446	850
8. Rabbits	1	0	95	0	95
9. Non-Human Primates	-	-	-	-	-
10. Sheep	-	-	-	-	-
11. Pigs	-	-	-	-	-
12. Other Farm Animals	-	-	-	-	-
13. Other Animals	-	-	-	-	-

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

SIGN[illegible]FICIAL	NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL *(Type or Print)*	DATE SIGNED
(b)(6),(b)(7)(c)	(b)(6),(b)(7)(c)	10-19-06

APHIS 18-23 (Oct 88), which is obsolete

(AUG 91)

PART 1 - HEADQUARTERS

OCT 23 2006

Colorado Serum Company
License No. 84-R-0007

For FY 2005-2006 Annual Report of Registered Research Facility

Each animal in each referenced test was employed in testing a Licensed Veterinary Biological product as required by Federal Regulations as codified in Title 9CFR. Humane euthanasia of animals on 9CFR required tests is provided for in 9CFR 117.4(e) - Animals at Licensed Establishments - Test Animals (filed August 19, 1995). This is included as standard testing protocol. While limiting the duration of the pain and distress, it does not fulfill the description of use as described treatment in Section D of APHIS Form 7023, Page 1. Therefore, we feel obligated to continue to include these test animals in Section E.

Attending veterinarians and related employees have been informed and Colorado Serum Company is complying with this provision.

Explanation of usage of animals listed in Column E.

Line Item 6. Guinea Pigs

1482 guinea pigs were tested causing pain, for which no anesthetic, analgesics, or tranquilizers could be used. A detailed breakdown of guinea pigs by test reference is provided.

9CFR 113.451(b) Tetanus Antitoxin potency testing - 1342 guinea pigs

Humane endpoints were addressed for all animals on this test, thus reducing the duration of pain and suffering.

9CFR 113.106(c) Clostridium Chauvoei Bacterin potency testing - 140 guinea pigs

Humane endpoints were addressed for all animals on this test, thus reducing the duration of pain and suffering.

Colorado Serum Company
License No. 84-R-0007

Line Item 7 Hamsters

446 hamsters were tested causing pain, for which no anesthetics, analgesics, or tranquilizers could be used. A detailed breakdown of hamsters by test referenced is provided.

9CFR 113.101(c) Leptospira Pomona Bacterin potency testing - 111 hamsters

The 111 hamsters included required controls for potency tests [9CFR 113.101(c)(2)], plus necessary hamsters required to establish the validity of the challenge dose (10 to 10,000 hamster LD_{50}) by titration. 9CFR 113.101 (c)(3).

For all test hamsters, Colorado Serum Company specifically has applied provisions of 9CFR 117.4(e) wherein ill animals were humanely euthanized upon reaching a point where death was expected to occur.

9CFR 113.102(c) Leptospira Icterohaemorrhagiae Bacterin potency testing - 99 hamsters

The 99 hamsters included required controls for potency tests [9CFR 113.102(c)(2)], plus necessary hamsters required to establish the validity of the challenge dose (10 to 10,000 hamster LD_{50}) by titration. 9CFR 113.102(c)(3).

For all test hamsters, Colorado Serum Company specifically has applied provisions of 9CFR 117.4(e) wherein ill animals were humanely euthanized upon reaching a point where death was expected to occur.

9CFR 113.103(c) Leptospira Canicola Bacterin potency testing - 106 hamsters

The 106 hamsters included required controls for potency tests [9CFR 113.103(c)(2)], plus necessary hamsters required to establish the validity of the challenge dose (10 to 10,000 hamster LD_{50}) by titration. (CFR 113.103(c)(3).

Colorado Serum Company
License No. 84-R-0007

Line Item 7	9CFR 113.103(c)	Continued For all test hamsters, Colorado Serum Company specifically has applied provisions of 9CFR 117.4(e) wherein ill animals were humanely euthanized upon reaching a point where death was expected to occur.
	9CFR 113.104(c)	Leptospira Grippotyphosa Bacterin potency testing - 130 hamsters The 130 hamsters included required controls for potency tests [(9CFR 113.104(c)(2)], plus necessary hamsters required to establish the validity of the challenge dose (10 to 10,000 hamster LD_{50}) by titration. 9CFR 113.104 (c)(3). For all test hamsters, Colorado Serum Company specifically has applied provisions of 9CFR 117.4(e) wherein ill animals were humanely euthanized upon reaching a point where death was expected to occur.

(b)(6),(b)(7)(c) _____ 10-19-06
Date

OCT 23 2006

COLORADO SERUM COMPANY

PHONE: (303) 295-7527
FAX: (303) 295-1923

(b)(2)High, (b)(7)(F) P.O. Box 16428 Denver, Colorado 80216-0428

July 22, 2004

Subject: Investigation by Colorado Serum Company's Institutional Animal Care and Use Committee into hamster analgesia and its possible usage in hamsters during the *Leptospira* challenge portion of *Leptospira* potency tests at Colorado Serum Company.

From: (b)(6),(b)(7)(c)

To: Colorado Serum Company Institutional Animal Care and Use Committee and Mr. (b)(6),(b)(7)(c) Colorado Serum Company.

Background: Use of hamsters for potency tests when developing Leptospira organisms for bacterin production has been the requirement by USDA (9-CFR, 113.101 – 113.105) for many years. Ten vaccinates and 10 or more controls are challenged intraperitoneally with a suspension of virulent Leptospira organisms. This applies to potency tests for *Leptospira pomona, Leptospira icterohaemorrhagiae, Leptospira canicola* and *Leptospira grippotyphosa* fractions. If 8 or more controls die from leptospirosis during a 14 day post challenge observation period then the test is considered valid. The degree of pain and suffering to the control hamsters is of concern for animal welfare reasons and an alternative in-vitro test that has reproducible and comparable results to the in-vivo potency test would be a much better alternative replacement test. Until that happens all biologic companies producing *Leptospira* bacterins will continue to use hamster in-vivo potency tests.

The degree of pain and suffering the control hamsters endure during this 14 day observation period is a subjective evaluation given the fact that hamsters cannot communicate with humans. Based on the disease syndrome of Leptospirosis in our domestic species the clinical signs can be mild to severe. In most animal species the disease causes septicemia, nephritis, depression and anorexia. It is safe to assume that hamsters suffer from many of these symptoms as well, and it is most likely not a chronic pain as much as it is a general discomfort or sick feeling that they are experiencing. Parameters of hamster behavior used to measure discomfort or pain would include exploration, grooming and posture as well as food and water consumption and fur quality.

Analgesic use in hamsters: An on-line and textbook search conducted by Colorado Serum Company's IACUC (b)(6),(b)(7)(c) revealed very few drug options for analgesia in hamsters (see attached documents). Buprenorphine was the most common analgesic for hamsters cited. The labeled route is by subcutaneous injection and the

frequency is every 8 hours. In one published paper (see attached) buprenorphine was used in the syngeneic murine tumor model to investigate whether the pain or discomfort in mice with these tumors could be reduced with this analgesic via an oral route. In this study they found no significant difference in pain or discomfort between the buprenorphine treated group and the control group based on their scoring system. Even though this experiment was done with mice it would be plausible to compare the discomfort or pain from multiple tumors to that which would be experienced by hamsters during a systemic leptospirosis infection. Also the process of handling these hamsters and giving them injections 2 – 3 times daily would itself be stressful and painful. There would be a significant zoonotic concern for the person involved giving the injections to multiple infected hamsters from the urine as well as an increased chance of being bitten considering you are giving injections to sick and uncomfortable hamsters 2 to 3 times daily for up to 14 days. Treating the symptoms without treating the source of the disease with antibiotics (which can't be done for obvious reasons) is also of questionable value. There would also be concern as to what effect this analgesic would have on the outcome of the potency test. All drugs are processed and eliminated by the liver and kidneys and since these are two target organs of *Leptospira* organisms, toxic levels of buprenorphine could buildup in a *Leptospira* infected hamster and could alter the time of death or even contribute to a death that might not otherwise happen - thus affecting the outcome of the potency test and possibly resulting in a falsely valid test. Other drugs mentioned are injectable also and do not offer anything unique or superior to buprenorphine.

The Cornell website detailed an oral preparation of buprenorphine in jello for use in mice (see attached). Whether this would work in hamsters is not known. One concern is the fact that anorexia is a very common symptom with Leptospirosis and depending on the degree of anorexia and the fact that these hamsters are usually housed 5 per cage, some hamsters may be getting their medication and some may not - due to anorexia or competition. This could also cause some hamsters to overdose on buprenorphine if they all aren't eating the same amount of jello.

Conclusion:
Colorado Serum Company's IACUC has determined that no analgesia we have found can realistically be expected to relieve an infected hamster from the symptoms of Leptospirosis especially without concurrent use of antibiotics to fight the etiologic agent (*Leptospira*) since this would defeat the purpose of the potency study. Buprenorphine works well with acute, sharp post-surgical type pain and would be of very questionable value in hamsters suffering from discomfort associated with a systemic infection. The best course of action in our opinion would be to replace the hamster in-vivo potency test with an in-vitro test that does not require animal testing for potency.

COLORADO SERUM COMPANY

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(b)(2)High, (b)(7)(F) P.O. Box 16428 Denver, Colorado 80216-0428

November 11, 2004

Subject: Investigation by Colorado Serum Company's Institutional Animal Care and Use Committee into guinea pig analgesia and its possible usage in guinea pigs during the *Clostridium tetani* antitoxin potency tests done at Colorado Serum Company.

From: (b)(6),(b)(7)(c)
Veterinarian.

To: Colorado Serum Company's Institutional Animal Care and Use Committee and Mr. (b)(6),(b)(7)(c) Colorado Serum Company.

Background: Use of guinea pigs for potency tests when developing Tetanus Antitoxin has been the requirement by USDA (9-CFR, 113.451) for many years. The potency test is essentially an in-vivo neutralization test that requires two guinea pigs each for controls and for each dilution. The two controls are injected subcutaneously with a 3 ml dose of the Standard Toxin-Antitoxin mixture. Injections shall be made in the same order that toxin is added to the dilutions of antitoxins. These shall be observed parallel with the titration of one or more unknown antitoxins. Two test guinea pigs will be used for each dilution of the unknown antitoxin (also a 3 ml dose, subcutaneously). Controls are observed until they are down and are unable to rise or stand under their own power. At this time they are humanely euthanized and the time of death is recorded in hours. For a satisfactory test, the controls must reach this point with clinical signs of tetanus within 24 hours of each other and within an overall time of 60 – 120 hours. The clinical signs to be observed are increased muscle tonus, curvature of the spine, asymmetry of the body outline when the resting animal is viewed from above, generalized spastic paralysis, particularly of the extensor muscles, inability to rise from a smooth surface when the animal is placed on its side, or any combination of these signs. If the control guinea pigs do not respond in this manner the entire test shall be repeated. Potency of an unknown antitoxin is determined by finding the mixture which will protect the test animal the same as the Standard Toxin-Antitoxin mixture. Test animals dying sooner than the controls indicate the unit value selected in that dilution was not present, whereas those living longer indicate a greater unit value.

Analgesia in Guinea Pigs: An on-line and textbook search conducted by Colorado Serum Company's IACUC (b)(6),(b)(7)(c) revealed a variety of drugs used in guinea pigs after surgical procedures. The Duke University IACUC website had a very thorough

guideline for analgesia in all rodents and a variety of drugs and routes of administration based on the degree of pain and discomfort of a given procedure (see attached). Injectable analgesic options for guinea pigs include Codeine, Nalbuphine or Morphine given every 4 hours or Buprenorphine every 12 hours beginning just before symptoms are estimated to begin. Oral medications include Aspirin or Phenylbutazone added to the drinking water once daily, however once guinea pigs begin to show signs of tetanus it can be expected that their water consumption will diminish greatly and that once they are prostrate they will physically be unable to stand and drink. At the later stages of tetanus they are unable to swallow. Euthanasia hopefully is done before the late stages of disease but unfortunately death is sometimes the outcome with the test pigs before the study investigator can officially call the test over, if they have a lower level of antibody protection when compared to the Standard Controls.

Conclusion: There is obviously a direct conflict between animal welfare issues (pain and suffering) and this neutralization potency test that is required by USDA-APHIS-CVB for each new serial of tetanus antitoxin. The dilemma is that the test relies solely on symptoms of tetanus in relation to the Standard Controls as described above, in essence pain and suffering are components of the measured parameters of the test and giving any drugs to alter or help alleviate the symptoms will affect the results of the test. Currently the best welfare that can be provided is humane euthanasia once the symptoms have reached the point that the study investigator can interpret the test and give the staff veterinarian the o.k. to intervene and euthanize.

Due to this direct conflict, Colorado Serum Company's Institutional Animal Care and Use Committee has determined that there is no practical way to intervene with pain medications during the Tetanus Antitoxin potency neutralization test without altering the animal symptoms and thus altering the interpretation of the test. The only solution to this is to replace the guinea pig test with an in-vitro test (which Colorado Serum Company would be greatly in favor of). Currently there is a Competitive Elisa Test for Tetanus Antitoxin that has been tried but unfortunately it has not shown consistent, comparable results when run in parallel with the guinea pig neutralization test.

(b)(6),(b)(7)(c)

November 12, 2004

COLORADO SERUM COMPANY
Established 1923

COLORADO
Veterinary Products

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Fax: (303) 295-1923

(b)(2)High, (b)(7)(F) P.O. Box 16428 - Denver, Colorado 80216-0428

October 19, 2006

Dr. Robert M. Gibbens, DVM
Director, Western Region Animal Care
USDA-APHIS-Animal Care
2150 Centre Avenue
Building B Mail Stop #36011
Ft. Collins, CO 80526

Dear Dr. Gibbens:

Enclosed please find our Research Facility Annual Report (APHIS Form 7023).

Supplementary documents are included which were enclosed with the 2005 Annual Report.

1. "Investigation into Guinea Pig Analgesia..... ", dated November 11, 2004.
2. "Investigation by Colorado Serum Company's IACUC into Hamster Analgesia..... ", dated July 22, 2004.

In each case we have found no new information which would allow a change in our approach to these issues.

We have also enclosed a description of "Symptoms in Control Guinea Pigs after Challenge with *Clostridium chauvoei*", dated December 19, 2004.

Throughout this year, provisions in 9CFR 117.4(e) were applied to all animals in tests conducted according to 9CFR 113.451(b), 9CFR 113.106(c), 9CFR 113.101(c), 9CFR 113.102 (c), 9CFR 113.103(c), and 9CFR 113.104(c).

In each test, guinea pigs or hamsters that showed clinical signs of illness due to the test were humanely destroyed if the illness progressed to the point when death was certain to occur without therapeutic intervention.

Regarding another matter, we request that you continue to use our P.O. Box 16428 on future inspection reports instead of our street address.

OCT 2 3 2006

Please contact us if there are any questions.

Sincerely,

JNH:al
Enclosure

December 29, 2004

(b)(6),(b)(7)(c)

Subject: **Symptoms in control guinea pigs after challenge with *Clostridium chauvoei* and *hemolyticum* during *Cl. chauvoei* and *hemolyticum* bacterin in vivo potency tests.**

These are symptoms that I have personally observed during this potency test. I will also include some more detailed textbook description of these symptoms to use if you like.

This potency tests for both of these fractions involves a 3 day observation after IM challenge with ~100 LD_{50} of live bacteria (*Cl. chauvoei or hemolyticum*). Within 24 to 48 hours the first symptoms of disease begin to appear. Lethargy, anorexia, stiffness with reluctance to move, sero-purulent discharge from the festering injection site, and vocalization (associated with pain) when handled are frequent symptoms most commonly seen. The swelling at the injection site is hot and painful at first and eventually becomes cold and necrotic as the infection becomes systemic. A high fever is also associated with these Clostridial diseases. Once symptoms are obvious the disease progresses quickly and death can happen rapidly. Once obvious symptoms are observed, veterinary intervention and humane euthanasia is implemented whenever possible.